PATENT
Docket no. 1173-1061PUS3

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: Peter L. COLLINS et al. Conf. No.: 5376

Appl. No.: 10/789, 400 Art Unit: 1632

Filed: Februrary 27, 2004 Examiner: Shin-Lin Chen

For: RECOMBINANT HUMAN METAPNEUMOVIRUS AND ITS USE

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Dr. Peter Collins, hereby declare as follows:

1. I am a U.S. citizen, residing at 2921 Woodstock Avenue, Silver Spring, MD 20910.

2. I am presently employed as Senior Investigator at the National Institutes of Allergy and Infectious Diseases of the National Institutes of Health. A copy of my Curriculum Vitae is attached.

3. I am a co-inventor of the subject matter of the above-identified U.S. Patent application. I am familiar with the specification and pending claims, and with the prosecution history of the application.

4. The Examiner has rejected claims 1-4, 6-8, 15, 16, 18, 25 55 and 56 of the application as being obvious in view of Bermingham et al., *Proc. Natl. Acad. Sci. USA,* 96:11259-11264 (1999)

in view of Van den Hoogen et al., *Nature Medicine* 7:719-714 (2001) and Van den Hoogen et al., *Virology* 295:119-132 (2002).

5. The Examiner asserts that Bermingham teaches the NdeI and K5 mutations, and a few others, that together are made to ablate expression of the M2 ORF2 of HRSV. The Examiner notes that Bermingham et al. use a "minigenome" comprising a Chloramphenicol Amino-Transferase ("CAT") gene under control of RSV transcription and termination signals to direct synthesis of CAT in the presence of RSV N, P and L proteins, thus as an indirect measure of RSV viral growth in culture. The Bermingham et al. reference is also described as showing that virus lacking M2-2 protein grew less efficiently in vitro and that the authors conclude that the M2-2 protein functions to switch the virus state between one of transcription of viral genes to replication of the viral genome.

6. The Van den Hoogen (2001) paper is cited as teaching the sequence of HMPV, an element necessary to provide enablement of the present invention and plainly lacking from the disclosure of Bermingham et al., which is directed to a virus distinct from HMPV.

7. The Van den Hoogen (2002) paper is cited as teaching that HMPV infection is a significant clinical problem, causes clinical symptoms similar to RSV, and like RSV is a member of the Pneumovirinae subfamily.

8. The Examiner concludes that one of ordinary skill in the art would have found it obvious, that is, a mere application of ordinary technical skill, to construct a recombinant HMPV or an expression vector having a partial or complete HMPV genome or antigenome comprising one or more attenuating modifications (as in claim 1 as filed), with a reasonable expectation of success in obtaining the present invention.

9. To the contrary, achieving the present invention required considerable inventive activity over what had been disclosed by Bermingham and Van den Hoogen.

10. Whereas RSV viruses can be recovered by reverse genetics methods and propagated in a wide number of cells of human, hamster, bovine, and simian origin, HMPV appears to replicate well only in two lines, namely African green monkey Vero cells and Rhesus monkey LLC-MK2 cells. This is unexpected and unexplained. Furthermore, HMPV replicates much more slowly than RSV, with an infectious cycle of 72-96 h or more compared to 24-48h for RSV. Final yields are reduced by approximately 10-fold or more, which further complicates studies. It is substantially less cytopathic, making it difficult to monitor growth. HMPV also depends on added trypsin in the medium for growth. Because trypsin is unstable due to self-cleavage and metabolism by the cell monolayer, one must first determine optimal conditions for growth and trypsin addition and re-addition, which vary with cell type. Because of the poor growth of HMPV, we had to develop the rescue system using a construct expressing green fluorescent protein as a living tag to monitor recovery and infection. We also could not use the traditional method of supplying T7 RNA polymerase with a vaccinia virus recombinant. This is because, given the long replication cycle and poor growth of HMPV, the rapidly growing vaccinia virus would kill the cells and preclude recovery. This is true even for attenuated strains such as the vaccinia MVA strain, since they remain very cytopathic compared to HMPV. For that reason, it became necessary to develop an amplification method in which we used an available baby hamster kidney cell line that constitutively produces T7 RNA polymerase for initial infection, and co-culture with susceptible Vero or LLC-MK2 cells. This allowed recovery of recombinant virus despite poor growth in the BHK cells. All of this required considerable experimentation and technical skill to finally achieve reliable recovery of recombinant HMPV from cDNA clones.

11. At the time the invention was made, there were reasons to be skeptical of RSV as an exact model for HMPV. The two viruses have been classified in different taxonomic genera, which is an unambiguous scientific determination that the viruses are substantially different. RSV has two additional genes (NS1 and NS2) compared to HMPV. There are some proteins that seem to be similar between the two viruses. However, the most similar ones (such as the

nucleocapsid protein N, phosphoprotein P, matrix protein M, and polymerase protein L) are present in a very wide array of viruses spanning four or more virus families involving widely different viral species. Thus, the presence of proteins bearing the same name is not necessarily indicative of close structural similarity or predictability of function. In addition to lacking the NS1 and NS2 proteins of RSV, HMPV has a different gene order for four of its eight genes compared to RSV. Since gene order is the single most conserved feature of the nonsegmented negative strand RNA viruses, this is indicative of significant difference between RSV and HMPV. Also while the disease caused by HMPV has some similarities to that of RSV, the virus infects later in infancy than RSV and elicits a very different host cytokine response.

12. Between HMPV and RSV, there is 36% amino acid identity between the two putative M2-1 proteins. This is a fairly low value, but the two proteins also share a cysteine-histidine motif that, for RSV, was shown to be important for M2-1 function. In contrast, there is only 12% identity for the two M2-2 proteins and an absence of any shared motif or conserved segment. A value of 12% is insignificant in the absence of conserved motifs, and hence there is no relatedness at all in M2-2 between HMPV and RSV. The M2-1 protein is essential for RSV replication: its deletion is lethal. However, even though M2-1 of HMPV has significant sequence relatedness with that of RSV, and shares a cysteine-histidine motif, we found that it was not essential for replication of HMPV. Specifically, HMPV from which the M2-1 coding sequence had been deleted replicated nearly as efficiently as wild type in cell culture. In addition, HMPV lacking both M2-1 and M2-2 replicated nearly as efficiently as wild type HMPV in cell culture. Thus, deletion of the M2-1 protein, which seems to be related between RSV and HMPV and in particular has a conserved cysteine-histidine motif known to be important for function in RSV, yielded results that were completely different than expectations. Since M2-2 had no sequence relatedness between the two viruses, and given the contrarian results with M2-1, it would not be reasonable to be able to anticipate results for M2-2. In other words, the contrarian results obtained with the M2-1 protein show that RSV and HMPV are significantly different – consistent with their classification into different genera - and that one cannot rely on a low level of

sequence relatedness or other vague similarities to make predictions between viruses from different taxonomic groups.

13. Other aspects of HMPV biology have proven to be different from RSV. For example, with RSV, the attachment G protein is a major neutralization and protective antigen, and is essential for replication in mice. In contrast, for HMPV, the G attachment protein is not a significant attachment or neutralization antigen and is not essential for replication in mice or in non-human primates. Furthermore, whereas RSV G has sequence relatedness to the CX3C chemokine called fractalkine, and mimics its chemotactic activity in vitro, there is no such sequence relatedness between HMPV G and any chemokine. In addition, the RSV G protein is expressed abundantly as a secreted form in addition to the membrane-anchored form. This secreted form functions as an antibody decoy to help the virus evade neutralizing antibodies and also shifts the polarization of T helper cells. Whereas the secreted form of G plays a central role in RSV biology, there is no known secreted from of HMPV G. This is another example involving a protein that seems somewhat similar between RSV and HMPV, but which turns out to have substantial functional differences and for which the effect of deletion is very different. In particular, the finding that G is a neutralization antigen for one virus (RSV) but not the other (HMPV) and is essential for detectable replication in vivo by one virus (RSV) but is dispensable and a useful method of attenuation for the other (HMPV) are major differences that substantially impact vaccine design. In this regard, if one assumed that RSV was a predictive model, one would have emphasized the use of G protein in any vaccine (and indeed at least commercial company has based an experimental RSV vaccine solely on G). Obviously, this presumption would have been calamitous for an HMPV vaccine program. This illustrates why experienced workers in the field recognize the need to evaluate each attenuating mutation made in HMPV as being novel.

14. At the present time, the M2-2 deletion in HMPV and RSV has some similarities but also has differences. Both yield viable virus. However, the kinetics of replication in vitro of the

RSV M2-2 deletion mutant were substantially reduced compared to wild type RSV, whereas the efficiency of replication of the HMPV M2-2 deletion mutant was the same or greater than that of wild type HMPV in Vero cells (that lack the type I interferon genes). That would be a very unexpected result for one anticipating that RSV would be an accurate predictive model for HMPV, and might suggest that the M2-2 deletion was not attenuating in HMPV. Transcription seems to be up-regulated in both viruses following deletion of M2-2. However, whereas the M2-2 deletion RSV has a decrease in RNA replication associated with the increase in transcription, there does not seem to be a decrease in RNA replication with HMPV. This indicates that the mechanism of the effect is not identical. In addition, there may be a slightly higher level of point mutations with deletion of M2-2 in HMPV compared with RSV, in particular ones in runs of A's and T's. Thus, M2-2 might have an effect on the fidelity of RNA synthesis for HMPV but not RSV. There also is preliminary evidence that M2-2 is an interferon antagonist in HMPV but not RSV (which has these functions in the NS1 and NS2 proteins that HMPV lacks). If so, this would be an important difference between an M2-2 deletion virus in HMPV versus RSV, since interferon affects immunogenicity in a positive way. In practical terms, clinical results and regulatory evaluation of an RSV vaccine based on deletion of M2-2 will not be considered in any way predictive of or relevant to an HMPV vaccine based on deletion of M2-2, given the differences between the viruses and the lack of confidence in extrapolations made across viral genera.

15. Although a partial HMPV sequence was available, this is insufficient to design recombinant virus or to develop vaccine strategies. As mentioned above, the partial sequence provided by Van den Hoogen did not necessarily contain all of the HMPV genes. The sequence provided by Van den Hoogen in fact lacked the key promoter sequences. It is not an authenticated, functional sequence, i.e., the sequence that was available had not been confirmed to encode a viable virus. This is essential given the high rate of mutation in RNA viruses. Our invention provides a complete functional sequence encoding a wild type-like virus and thus provides a working copy. In other words, one can construct a clone of that sequence and be

assured that it will produce infectious virus. Our invention also establishes which proteins must be expressed in trans for recovery – this complement of proteins turns out to be different from RSV since M2-1 is not needed for HMPV replication. As already noted above, our invention also describes precise methods for recovery.

16. In summary, there are substantial differences in the biology and biochemistry of replication of HMPV compared to RSV. These differences are not known in full, since HMPV is a newly described virus and both viruses continue to be the subject of intense research. However, the differences identified to date are sufficient that the two viruses are classified in different genera. A number of specific and unexpected differences have been noted above. Contrary to the assertion of the Examiner, one of ordinary skill in the art, having before him the teachings of Bermingham regarding RSV and the sequence data and clinical information from Van den Hoogen, would **not** have a reasonable expectation of success in achieving an attenuated HMPV (claim 1) by applying the techniques of Bermingham to HMPV to make a recombinant virus that did not express a functional M2-2 protein. Some of the obstacles involve the lack of a working HMPV model, including the lack of a complete inventory of the genes, the lack of information on the specific functions of the genes, the lack of a complete confirmed sequence, and the lack of methods for recovering this slow growing and fastidious virus by recombinant methods. Other obstacles involve differences, both known and anticipated, between the viruses that preclude direct extrapolation between distinct taxonomic groups. Above, we have given examples of substantial differences in the properties of the M2-1, M2-2, and G proteins and viruses from which these genes have been deleted. Pertinent to the M2 gene, HMPV lacking functional M2-2 protein is not attenuated in cells that do not produce interferon, whereas RSV lacking functional M2-2 protein is highly attenuated in vitro regardless of interferon production. The Examiner is referred to pp. 79-80 of the specification. This is consistent with function of the M2 proteins of HMPV as interferon antagonists. With regard to M2-1, deletion of this protein from RSV is lethal, consistent with its role as an essential transcription elongation factor. In contrast, deletion of M2-1 from HMPV is only slightly attenuating in vitro and yields a virus that appears to transcribe without impediment. It is clear that

one cannot extrapolate between these two viruses. The Examiner's speculation that one could directly apply the knowledge about how to attenuate RSV to HMPV is only that. Absent the teachings of the present specification, there simply was not enough known about HMPV at the time the present invention was made to make attenuated HMPV viruses in a directed, predicable fashion using genetic engineering methods.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:_____ By_____

(Dr. Peter Collins)